Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	627	carboxylesterase$1	US-PGPUB; USPAT	OR	OFF	2005/05/02 07:51
L2	12751	cpt-11 or (cpt adj "11") or apc	US-PGPUB; USPAT	OR	OFF	2005/05/02 07:51
L3	3172	camptothecin	US-PGPUB; USPAT	OR	OFF	2005/05/02 07:52
L4	174	1 same (2 or 3)	US-PGPUB; USPAT	OR	OFF	2005/05/02 07:52
L5	38	rabbit same 1	US-PGPUB; USPAT	OR	OFF	2005/05/02 07:53
L6	13	4 and 5	US-PGPUB; USPAT	OR	OFF	2005/05/02 07:53

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TITLE: Microorganisms and cells for diagnosis and therapy of tumors

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INVENTOR-INFORMATION:

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EP	011184173	2001EP-011184173	July 31, 2001
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US-CL-CURRENT: 424/1.11, 424/9.3 , 424/9.6 , 424/93.21 , 514/44

ABSTRACT:

Described are diagnostic and pharmaceutical compositions comprising a microorganism or cell containing a DNA sequence encoding a detectable protein or a protein capable of inducing a detectable signal, e.g. a luminescent or fluorescent protein, and, in a particular embodiment, furthermore (a) DNA sequence(s) encoding (a) protein(s) suitable for tumor therapy and/or elimination of metastatic tumors, e.g. a cytotoxic or cytostatic protein.

---------- KWIC ---------

Summary of Invention - Table CWU - BSTL (7):
4TABLE 4 Examples of enzyme/prodrug pairs in which the delivery of the enzyme gene is facilitated by intravenously injected microorganisms and cells: Therapeutic proteins (enzymes), drugs, prodrugs Description References Herpes simplex virus Most well-known Moolten Cancer Res. thymidine kinase (HSV- enzyme/produrg combination. 1986; 46: 5276-5281 TK) +Ganciclovir (GCV) Herpes simplex virus thymidine A-5021 has highly selective antihepatic Hasegawa et al. 2000, kinase (HSV-TK) activity and was selectively Cancer Gene

+A-5021 (1'S,2'R)- phosphorylated by viral TK Ther 7: 557-562 9[[1',2'- in herpes virus-infected cells. bis(hydroxymethyl)cycloprop- The anti-herpetic activity of A- 1'-yl]methyl]guanine 5021 was most potent in comparison with ACV and penciclovir. Horseradish peroxidase When activated by purified Greco et al. 2000, Cancer Gene (HRP) HRP, IAA was shown to Thera. 7: 1414-1420 +Indole-3-acetic acid inhibit colony formation in (IAA) mammalian cells, whereas, neither enzyme nor prodrug alone was cytotoxic at the same concentration or times. The HRP/IAA-induced cell kill was effective in normoxic and anoxic conditions. Bacterial enzyme carboxypeptidase CPG2 can be expressed both Spooner et al. 2000, G2 (CPG2) intracellularly or tethered to Cancer Gene +4-([2-chloroethyl][2- the outer surface of Ther. 7: 1348-1356 mesyloxyethyl]amino)benzoyl- mammalian cells, where it is Webley et al. 2001, L-glutamic acid able to activate mustard Br J Cancer 84: (CMDA) prodrugs for use in suicide 1671-1676 or +4-[N,N-bis(2- gene therapy. iodoethyl) amino] phenoxycarbonyl L-glutamic acid (ZD2767P) Human cytochrome P450 Acetaminophen is cytotoxic Thatcher et al. 2000, CYP1A2 through the cytochrome P450- Cancer Gene +acetaminophen mediated generation of a Ther 7: 521-525 chemically reactive metabolite, N-acetylbenzoquinoneimine (NABQI). Rabbit cytochrome P450 CYP4B1 is able to induce Mohr et al. 2000, Cancer Gene Ther. 4B1 (CYP4B1) tumor cell death at low micro- 7: 1008-1014; +4-ipomeanol (4-IM) molar concentrations in Heuser et al. 2000, Cancer Gene glioblastoma cells after treatment Ther 7: 806-12 with the prodrug 4-IM. Rat cytochrome P450 4BI The CYP2B1 gene product Kammertoens et al. 2000, Cancer (CYP2B1) activates oxaphosphorines to Gene Ther 7: 629-636 +oxaphosporines, such as the hydroxy form, giving rise ifosfamide (IFO) to the toxic products phosphamide mustard and acrolein, which alkylate DNA and proteins, respectively. E. coli nitroreductase CB1954 is a weak Djeha et al. 2000, Cancer Gene (NTR) monofunctional alkylating Ther. 7: 721-731; +CB1954 agent that is converted by NTR Djeha et al. 2001, Mol Ther 3: 233-240 into 2- and 4-hydroxylamino Westphal et al. 2000, Cancer Gene derivatives. Cellular thioesters Ther 7: 97-106 such as acetyl coenzyme A Weedon et al. 2000, Int J Cancer subsequently convert the latter 86: 848-854 into a powerful bifunctional alkylating agent that can kill both proliferating and nonproliferating cells. PTX0147 is the plasmid expressing NTR from the human cytomegalovirus (CMV) early promoter/ enhancer and also carries the b-globin second intron and poly (A) sequences and a G418 selectable marker. E. coli cytosine deaminase Despite CD expression, a Koyama et al. 2000, Cancer Gene (CD), E. coli uracil number of tumor cells were 5- Ther. 7: 1015-1022; phosphoribosyltransferase FC-resistant, which may be Theys et al. 2001, Cancer Gene Ther (UPRT) attributable to the lack of an 8: 294-297; active cytosine transport Kammertoens et al. 2000, Cancer +5-fluorocytosine (5-FC) system in mammalian cells and Gene Ther 7: 629-636; to the degradation of the Block et al. 2000, Cancer Gene Ther formed 50FU by 7: 438-445; dihydropyrimidine Bentires-Alj et al. 2000 Cancer Gene dehydrogenase (DPD). In the Ther 7: 20-26; gene transfer strategy, to improve Kawamura et al. 2000, Cancer Gene the effect of the CD/5- Ther 7: 637-643; FC system, it might be possible Li et al. 1997, Cancer Gene Ther to transduce the enzyme gene 4: 113-117 that converts 5-FU to its active forms. One of the candidates is E. coli UPRT. It is a pyrimidine salvage enzyme and is characteristic to bacteria. It directly converts 5-FU to 5- fluorouridine monophosphate (FUMP) at the first step of 5- FU activation and has the potential to enhance the activating pathway against DPD. Cytochrome P450 enzymes Liver tissue has a high content Huang et al. 2000, Cancer Gene +cyclophosphamide of P450 enzymes active toward Ther. 7: 1034-1042; (CPA) CPA and is the major organ Kan et al. 2001, Cancer Gene Ther responsible for CPA activation. 8: 473-482 Activated CPA generated in the liver circulates through the blood and gains entry to tumor tissue to exert its therapeutic effects. Intratumoral CPA activation can result in a high, localized concentration of active drug metabolite at its site of action, which may maximize therapeutic effects while at the same time

minimizing the host toxicities associated with hepatic drug activation.
rabbit carboxylesterase Exposure of neuroblastoma Meck et al. 2001, Cancer Res
+7-ethyl-10-[4-(1-piperidino)- cell lines or of mixtures of 61: 5083-5089
1-piperidino] these cell lines with CD34(+) carbonyloxycamp- cells at a ratio
of 10:90 to tothecin (CPT-11) replication-deficient AdRSVrCE for 24 h and
subsequent exposure of cells to 1-5 microM CPT-11 for 4 h increased the
toxicity of CPT- 11 to three Neuroblastoma cell lines (SJNB-1, NB-1691, and
SK- N-SH) from approximately 20-50- fold and eradicated their clonogenic
potential. Mushroom tyrosinase A sterically undemanding Jordan et al 2001,
Bioorg Med Chem +bis-(2-chloroethyl)amino- prodrug bis-(2-chloro- 9:
1549-1558 4-hydroxyphenylaminomethanone ethyl)amino-4-hydroxypheny-
laminomethanone 28 28 was synthesised and found to be oxidised by mushroom
tyrosinase at a superior rate to tyrosine methyl ester, the carboxylic acid
of which is the natural substrate for tyrosinase. E. coli
.beta.-galactosidase Prodrug cleaved by Tietze et al. 2001, Chembiochem
+1-chloromethyl-5- galactosidase shows high 2: 758-765 hydroxy-1,2-dihydro-3H
cytotoxicity towards human benz[e]indole (CC-1065) bronchial carcinoma cells
of or +1-(1'-chloroethyl)-5- line A549. hydroxy-1,2-dihydro-3H-
benz[e]indole A mutant of carboxypeptidase Activation of all three of the
Friedlos et al. 2002, Cancer Res G2 (CPG2, glutamate prodrugs not only kills
the cells 62: 1724-1729 carboxypeptidase expressing the mutant CPG2
+4-[bis(2- on the surface but also the iodoethyl)amino]- neighboring cells
through by- phenyloxycarbonyl-L-glutamic stander effect. acid or
+3-fluoro-4-[bis(2- chlorethyl)amino]benzoyl- L-glutamic acid or
+3,5-difluoro-4-[bis(2- iodoethyl)amino]benzoyl- L-glutamic acid Note: The
content of this table is by no-means to be exhaustive. Any other similar
enzyme-prodrug pairs, which are not listed in this table, are also considered
to be included.

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TITLE: Carboxylesterase inhibitors

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INVENTOR-INFORMATION:

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APPL-NO: 10/ 925367

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RELATED-US-APPL-DATA:

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US-CL-CURRENT: 514/357, 514/408 , 514/617 , 514/621

ABSTRACT:

This disclosure relates to amides, aryl sulphonamides, aryl ureas, and .alpha.,.beta.-diketones derivatives useful as carboxylesterase esterase inhibitors. The disclosure is also directed to the use of these compounds as selective human intestinal carboxylesterase inhibitors and insect carboxylesterase inhibitors. The disclosure is also directed to pharmaceutical compositions and pesticide formulations containing these compounds, and to methods for treating or ameliorating the toxic effects following administration of drugs such as cancer therapy drugs, treating or ameliorating the effects of a drug overdose, and to the use of the compounds for increasing the effectiveness of insecticides and pesticides.

[0001] This application claims priority under 35 U.S.C. .sctn.119(e) to U.S. Provisional Application Ser. No. 60/498,778, filed Aug. 29, 2003, which is incorporated by reference herein in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):

[0005] As yet, no endogenous substrates for CEs have been identified, although they are responsible for the metabolism of many drugs, including CPT-11, cocaine, heroin, meperidine, and capecitabine. These carboxylesterase enzymes are processed in the endoplasmic reticulum of mammalian cells, and hence these proteins can be secreted into the extracellular milieu. Recently, the x-ray crystal structure of a rabbit-liver and a human-liver carboxylesterase have been determined. These studies indicate that the

proteins demonstrate similar structures to other esterases including acetylcholinesterases, lipases, etc.

Summary of Invention Paragraph - BSTX (7):
[0007] 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (Irinotecan, CPT-11) is a widely used anti-cancer drug that has demonstrated remarkable promise in the treatment of solid tumors. CPT-11 has demonstrated remarkable antitumor activity in both preclinical models and patients with refractory disease and, as such, has recently been approved for the treatment of colon cancer in adults. When administered to patients, CPT-11 is activated by human carboxylesterase to yield its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), which is a potent topoisomerase I poison. Topoisomerases are the enzymes responsible for unwinding and winding chromosomal DNA. In order to allow transcription and translation, DNA must be unwound. SN-38 prevents DNA unwinding, and thus inhibits critical cellular processes in tumor cells, resulting in cell death.

Summary of Invention Paragraph - BSTX (12):
[0012] Recently, a rabbit liver carboxylesterase that could efficiently convert CPT-11 to SN-38 was isolated. A human homolog of this carboxylesterase (hCE1) is known. However, expression of hCE1 in human tumor cells does not alter their sensitivity to CPT-11. More recently, it has been demonstrated that both the human and mouse small intestine expresses high levels of carboxylesterases that can convert CPT-11 to SN-38. A cDNA encoding a human small intestinal carboxylesterase (hiCE) has subsequently been identified that is highly efficient at activating CPT-11. Expression of this protein in mammalian cells sensitizes them to the drug.

Summary of Invention Paragraph - BSTX (14):
[0014] There is therefore a need to develop new compounds that are not only useful as general esterase inhibitors, but further, to develop new compounds that are specific for the inhibition of selected carboxylesterases, such as the human small intestine carboxylesterases (hiCEs) that activate drugs such as CPT-11.

Detail Description Paragraph - DETX (4):
[0026] CPT-11 is an anti-cancer drug that is selectively hydrolyzed to SN-38 by a human carboxylesterase. SN-38 is a potent topoisomerase I inhibitor. One of the major problems associated with CPT-11 administration is gastrointestinal toxicity, such as delayed diarrhea, due to activation of CPT-11 by carboxylesterases in the human intestine. By administering the selective hiCE inhibitors of Formula (I)-(V), the conversion of CPT-11 to the active metabolite SN-38 in the gut is minimized, and hence CPT-11-induced gastrointestinal toxicity is ameliorated.

Detail Description Paragraph - DETX (70):
[0081] A preferred mode of administration of the specific inhibitors of the human intestinal carboxylesterase, identified in Table 1, is oral administration. When administered orally, these poorly bioavailable molecules are expected to either remain in the gut or only enter the epithelia of the lining of the intestine, and hence inactivate any carboxylesterase in these tissues, thus preventing subsequent activation of CPT-11 that is deposited in the duodenum from the bile.

Detail Description Paragraph - DETX (73):
[0084] Formulations comprising a compound of Formula (I)-(V) and a drug, for example, CPT-11, which is metabolized by a carboxylesterase to generate, for example, a topoisomerase I inhibitor, are also envisaged.

Detail Description Paragraph - DETX (85):

[0093] Ki values, determined for human small intestine carboxylesterase (hiCE), human liver carboxylesterase (hCE1), rabbit liver carboxylesterase (rCE), human acetylcholinesterase (hAcCHE), and human butyrylcholinesterase (hBuChE) for the selective human intestinal (hiCE) carboxylesterase inhibitors identified in Table 3 are set forth in Table 5:

Detail Description Paragraph - DETX (87):

[0095] Ki values, determined for human small intestine carboxylesterase (hiCE), human arboxylesterase (hCE1), rabbit liver carboxylesterase (rCE), human acetylcholinesterase (hAcCHE), and human butyrylcholinesterase (hBuChE) for other carboxylesterase inhibitors of the present invention are set forth in Table 6:

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DOCUMENT-IDENTIFIER: US 20050002945 A1

TITLE: Therapeutic and diagnostic conjugates for use with multispecific antibodies

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INVENTOR-INFORMATION:

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parent continuation-in-part-of 09337756 19990622 US PENDING

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non-provisional-of-provisional 60090142 19980622 US

non-provisional-of-provisional 60104156 19981014 US

US-CL-CURRENT: 424/184.1, 530/403

ABSTRACT:

Disclosed are compounds that include two or more haptens conjugated by a spacer or a carrier. The haptens may include diethylenetriaminepentaaceta- te (DTPA),

histimine-succinyl-glutamine (HSG), or combinations of DTPA and HSG. The compound also includes an effector molecule which may be conjugated to one or more of the haptens, the spacer/carrier, or both. The effector molecule may be conjugated by a number of linkages including an ester linkage, an imino linkage, an amino linkage, a sulfide linkage, a thiosemicarbazone linkage, a semicarbazone linkage, an oxime linkage, an ether linkage, or combinations of these linkages. Also disclosed are methods of synthesizing the compounds and/or precursors of the compounds.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. application Ser. No. 10/150,654, filed May 17, 2002; which is a continuation-in-part of U.S. application Ser. No. 09/382,186, filed Aug. 23, 1999 and a continuation-in-part of U.S. application Ser. No. 09/823,746, filed Apr. 3, 2001; both of which are continuations-in-part of U.S. application Ser. No. 09/337,756, filed Jun. 22, 1999; which claims the benefit under 35 U.S.C. .sctn. 119(e) to U.S. Application No. 60/090,142, filed Jun. 22, 1998, and to U.S. Application No. 60/104,156, filed Oct. 14, 1998. The contents of all the aforementioned applications are incorporated herein by reference in their entireties.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (21):
[0020] In one embodiment, the effector molecule may be a prodrug that is activated after the compound is administered to a subject. For example, a prodrug may be activated after it is localized to a targeted cell and/or internalized by the targeted cell. In particular, the prodrug may be activated by physiological conditions in the cell (e.g., the acidic environment of lysosomes). Alternatively, the prodrug may be activated by one or more enzymes, (e.g., carboxylesterase can activate prodrugs such as irinotecan (CPT-11). Preferably, the effector molecule includes camptothecin, doxorubicin, or derivatives and/or analogs thereof, and preferably the effector molecule is conjugated by an ester linkage. Doxorubicin derivatives and/or analogs include 2-pyrrolinodoxorubicin (2P-DOX) and cyano-morpholino doxorubicin.

Detail Description Paragraph - DETX (48):
[0114] The prodrug CPT-11 (irinotecan) is converted in vivo by carboxylesterase to the active metabolite SN-38. One application of the therapeutic method, therefore, is to use a bsAb targeted against a tumor and a hapten (e.g. di-DTPA) followed by injection of a di-DTPA-carboxylesterase conjugate. Once a suitable tumor-to-background localization ratio has been achieved, the CPT-11 is given and the tumor-localized carboxylesterase serves to convert CPT-11 to SN-38 at the tumor. Due to its poor solubility, the active SN-38 will remain in the vicinity of the tumor and, consequently, will exert an effect on adjacent tumor cells that are negative for the antigen being targeted. This is a further advantage of the method. Modified forms of carboxylesterases have been described and are within the scope of the disclosed compounds and methods. See, e.g., Potter et al., Cancer Res. 58:2646-2651 (1998) and Potter et al., Cancer Res. 58:3627-3632 (1998). In another embodiment, CPT-11 may be conjugated to a targetable construct that includes DTPA or a targeting molecule, which can further enhance localization and activation of CPT-11 to SN-38 at the tumor.

Detail Description Paragraph - DETX (49):
[0115] Etoposide is a widely used cancer drug that is detoxified to a major

extent by formation of its glucuronide and is within the scope of the disclosed compounds and methods. See, e.g., Hande et al. Cancer Res. 48:1829-1834 (1988). Glucuronide conjugates can be prepared from cytotoxic drugs and can be injected as therapeutics for tumors pre-targeted with mAb-glucuronidase conjugates. See, e.g., Wang et al. Cancer Res. 52:4484-4491 (1992). Accordingly, such conjugates also can be used with the pre-targeting approach described here. Similarly, designed prodrugs based on derivatives of daunomycin and doxorubicin have been described for use with carboxylesterases and glucuronidases. See, e.g., Bakina et al. J. Med Chem. 40:4013-4018 (1997). Other examples of prodrug/enzyme pairs that can be used within the present methods include, but are not limited to, glucuronide prodrugs of hydroxy derivatives of phenol mustards and beta-glucuronidase; phenol mustards or CPT-11 and carboxypeptidase; methotrexate-substituted alpha-amino acids and carboxypeptidase A; penicillin or cephalosporin conjugates of drugs such as 6-mercaptopurine and doxorubicin and beta-lactamase; etoposide phosphate and alkaline phosphatase.

Detail Description Paragraph - DETX (216):

[0217] Two vials of rabbit liver carboxylesterase (SIGMA; protein content .about.17 mg) are reconstituted in 2.2 ml of 0.1 M sodium phosphate buffer, pH 7.7 and mixed with a 25-fold molar excess of CA-DTPA using a freshly prepared stock solution (.about.25 mg/ml) of the latter in DMSO. The final concentration of DMSO in the conjugation mixture is 3% (v/v). After 1 hour of incubation, the mixture is pre-purified on two 5-mL spin-columns (Sephadex G50/80 in 0.1 M sodium phosphate pH 7.3) to remove excess reagent and DMSO. The eluate is purified on a TSK 3000G Supelco column using 0.2 M sodium phosphate pH 6.8 at 4 ml/min. The fraction containing conjugate is concentrated on a Centricon-10.TM. concentrator, and buffer-exchanged with 0.1 M sodium acetate pH 6.5. Recovery: 0.9 ml, 4.11 mg/ml (3.7 mg). Analytical HPLC analysis using standard conditions, with in-line UV detection, revealed a major peak with a retention time of 9.3 min and a minor peak at 10.8 min in 95-to-5 ratio. Enzymatic analysis showed 115 enzyme units/mg protein, comparable to unmodified carboxylesterase. Mass spectral analyses (MALDI mode) of both unmodified and DTPA-modified CE shows an average DTPA substitution ratio near 1.5. A metal-binding assay using a known excess of indium spiked with radioactive indium confirmed the DTPA:enzyme ratio to be 1.24 and 1.41 in duplicate experiments. Carboxylesterase-DTPA is labeled with In-111 acetate at a specific activity of 12.0 mCi/mg, then treated with excess of non-radioactive indium acetate, and finally treated with 10 mM EDTA to scavenge off excess non-radioactive indium. Incorporation by HPLC and ITLC analyses is 97.7%. A HPLC sample is completely complexed with a 20-fold molar excess of bi-specific antibody hMN-14 Fab'.times.734 Fab', and the resultant product further complexes with WI2 (anti-ID to hMN-14), with the latter in 80-fold molar excess with respect to bi-specific antibody.

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TITLE: Compositions and methods for sensitizing and inhibiting growth of human tumor cells

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INVENTOR-INFORMATION:

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non-provisional-of-provisional 60075258 19980219 US

US-CL-CURRENT: 514/44, 435/197 , 435/320.1 , 435/366 , 435/455 , 536/23.2

ABSTRACT:

Polynucleotides encoding carboxylesterase enzymes and polypeptides encoded by the polynucleotides which are capable of metabolizing a chemotherapeutic prodrug and inactive metabolites thereof to active drug are provided. Compositions and methods for sensitizing tumor cells to a prodrug chemotherapeutic agent and inhibiting tumor growth with this enzyme are also provided. In addition, screening assay for identification of drugs activated by this enzyme are described.

[0001] This application is a continuation of U.S. application Ser. No. 09/595,682 filed Jun. 16, 2000, which is a continuation-in-part of PCT/US99/03171 filed Feb. 12, 1999, which claims the benefit of priority from provisional U.S. application Ser. No. 60/075,258, filed Feb. 19, 1998.

[0002] This invention was supported in part by funds from the U.S. Government NIH Grant Nos. CA-66124 and CA-63512 and the U.S. Government may therefore have certain rights in the invention.

---------- KWIC --------

Summary of Invention Paragraph - BSTX (8):

[0008] CPT-11 (irinotecan, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin) is a prodrug currently under investigation for the treatment of cancer that is converted to the active drug known as SN-38 (7-ethyl-10-hydroxy-camptothecin) (Tsuji, T. et al. 1991. J. Pharmacobiol. Dynamics 14:341-349; Satoh, T. et al. 1994. Biol. Pharm. Bull. 17:662-664). SN-38 is a potent inhibitor of topoisomerase I (Tanizawa, A. et al. 1994. J. Natl. Cancer Inst. 86:836-842; Kawato, Y. et al. 1991. Cancer Res. 51:4187-4194), an enzyme whose inhibition in cells can result in DNA damage and induction of apoptosis (Hsiang, Y.-H. et al. 1989. Cancer Res. 49:5077-5082). The specific enzyme responsible for activation in vivo of CPT-11 has not been identified, although serum or liver homogenates from several mammalian species have been shown to contain activities that convert CPT-11 to SN-38 (Tsuji, T. et al. 1991. J. Pharmacobiol. Dynamics 14:341-349; Senter, P. D. et al. 1996. Cancer Res. 56:1471-1474; Satoh, T. et al. 1994. Biol. Pharm. Bull. 17:662-664). Uniformly, these activities have characteristics of carboxylesterase (CE) enzymes (Tsuji, T. et al. 1991. J. Pharmacobiol. Dynamics 14:341-349; Senter, P. D. et al. 1996. Cancer Res. 56:1471-1474; Satoh, T. et al. 1994. Biol. Pharm. Bull. 17:662-664). In fact, SN-38 can be detected in the plasma of animals and humans minutes after the administration of CPT-11 (Stewart, C. F. et al. 1997. Cancer Chemother. Pharmacol. 40:259-265; Kaneda, N. et al. 1990. Cancer Res. 50:1715-1720; Rowinsky, E. K. et al. 1994. Cancer Res. 54:427-436), suggesting that a CE enzyme present in either serum or tissues can convert the camptothecin analog to its active metabolite.

Summary of Invention Paragraph - BSTX (17):

[0017] In the present invention, polynucleotides encoding carboxylesterase enzymes or active fragments thereof and polypeptides encoded thereby which are capable of metabolizing the chemotherapeutic prodrug CPT-11 and its inactive metabolite APC to active drug SN-38 are disclosed. Use of these enzymes in combination with APC renders this inactive metabolite a useful chemotherapeutic prodrug. It has also been found that compositions comprising a polynucleotide of the present invention and a disease-specific responsive promoter can be delivered to selected tumor cells to sensitize the tumor cells to the chemotherapeutic prodrug CPT-11, thereby inhibiting tumor cell growth.

Brief Description of Drawings Paragraph - DRTX (2):

[0027] FIG. 1 shows the alignment of the amino acid sequences of a rabbit liver carboxylesterase (Rab; GenBank Accession # AF036930), a human liver carboxylesterase (hCE1; GenBank Accession # M73499) and the human intestinal carboxylesterase (hiCE; GenBank Accession # Y09616). The active site triad (Ser-240, Glu-364 and His-478) are indicated by an asterisk (*). Identical residues are indicated by a vertical line (.vertline.), conservative changes by a colon (:), semi-conservative changes by a period (.), and computer inserted gaps within the amino acids are indicated by a dash (-). Large areas of homology between all three proteins are shaded.

Brief Description of Drawings Paragraph - DRTX (5):

[0030] FIG. 4 shows the complete coding sequence of the rabbit liver CE (SEQ ID NO:20) and the amino acid sequence encoded thereby (SEQ ID NO:21). The 1698 bp ORF encodes a 62.3 kDa protein. The N-terminal hydrophobic leader sequence is in italics, the 5' and 3' RACE sequences are underlined and the potential active site serine is indicated by an asterisk. The carboxylesterase B-1 and

B-2 motifs, at amino acids 208-223 and 114-124 are double underlined. Numbers over the sequence refer to nucleotide position whereas numbers along the left margin refer to amino acid residues.

Detail Description Paragraph - DETX (2):

[0038] CPT-11 is a promising anti-cancer prodrug, that when given to patients, is converted to its active metabolite SN-38 by a human carboxylesterase. However, conversion in patients is relatively inefficient and less than 5% of the prodrug is metabolized to SN-38 (Rivory, L. P. et al. 1997. Clin. Cancer Res. 3:1261-1266). In patients, this prodrug is also metabolized to APC (Haaz, M-C. et al. 1998. Cancer Res. 58:468-472). APC has little, if any, active anti-tumor activity and is not converted to an active metabolite in humans (Rivory, L. P. et al. 1996. Cancer Res. 56:3689-3694). Accordingly, high concentrations of this prodrug must be administered to achieve effective levels of active drug in vivo. However, myelosuppression and secretory diarrhea limit the amount of prodrug that can be administered to patients.

Detail Description Paragraph - DETX (4):

[0040] In accordance with one aspect of the present invention there are provided polynucleotides which encode carboxylesterases capable of metabolizing a chemotherapeutic prodrug and inactive metabolites thereof to active drug. By "polynucleotides" it is meant to include any form of DNA or RNA such as cDNA or genomic DNA or mRNA, respectively, encoding these enzymes or an active fragment thereof which are obtained by cloning or produced synthetically by well known chemical techniques. DNA may be double- or single-stranded. Single-stranded DNA may comprise the coding or sense strand or the non-coding or antisense strand. Thus, the term polynucleotide also includes polynucleotides which hybridize under stringent conditions to the above-described polynucleotides. As used herein, the term "stringent conditions" means at least 60% homology at hybridization conditions of 60.degree. C. at 2.times.SSC buffer. In one embodiment, the polynucleotide comprises the cDNA depicted in FIG. 4 (SEQ ID NO:20) or a homologous sequence or fragment thereof which encodes a polypeptide having similar activity to that of this rabbit liver CE enzyme. In another embodiment, the polynucleotide comprises a cDNA as depicted in SEQ ID NO:27 encoding human intestinal carboxylase as depicted in SEQ ID NO:28. Due to the degeneracy of the genetic code, polynucleotides of the present invention may also comprise other nucleic acid sequences encoding these enzymes and derivatives, variants or active fragments thereof. The present invention also relates to variants of these polynucleotides which may be naturally occurring, i.e., allelic variants, or mutants prepared by well known mutagenesis techniques.

Detail Description Paragraph - DETX (24):

[0060] Another aspect of the present invention relates to the ability of compositions comprising a polynucleotide encoding a carboxylesterase and a disease-specific responsive promoter of selected tumor cells to sensitize the tumor cells to a chemotherapeutic prodrug. The ability of a rabbit CE or a human intestinal CE of the present invention to sensitize human tumor cells to CPT-11 was examined. Experiments were first performed to confirm that the metabolite produced by the activity of a CE of the present invention is biologically active in vitro. Rh30 cells were exposed to the products of each reaction for one hour and the percentage of growth inhibition was determined. As expected, Rh30 cells exposed to 1 to 5 units of CE that had been inactivated by heating produced no inhibition of cell growth. In contrast, reaction products of CPT-11 incubated with 1 to 5 units of active CE produced a 30-60% inhibition of cell growth. These data are consistent with the conversion of CPT-11 to SN-38 by CE in these cells. Similar confirmatory experiments were performed with COS-7 cells.

Detail Description Paragraph - DETX (43):

[0076] The rabbit proteins were subjected to automated N-terminal amino acid sequencing. Both bands yielded protein sequences indicating that the peptides were not N-terminally blocked. The derived amino acid sequences were analyzed by computer searches using the Fasta and BLAST comparison programs. Band 1 (approximately 60 kDa) demonstrated significant homology with several CE sequences, including a rabbit CE, present in the GenBank and Swissprot databases (FIG. 1). However, the nucleic acid sequence encoding rabbit CE protein has not been disclosed. In addition, comparison of the amino acid sequence of the polypeptide encoded by the cDNA of the present invention with the published amino acid sequence for rabbit CE showed three mismatches. Further, the polypeptide encoded by the cDNA of the present invention contains an 8 amino acid insert and an 18 amino acid leader sequence at the N-terminus which the published sequence does not contain. Thus, the published amino acid sequence of a rabbit liver carboxylesterase protein (Swissprot Accession Number P12337; Korza, G. and J. Ozols. 1988. J. Biol. Chem. 263:3486-3495) is different from the polypeptide encoded by the cDNA of the present invention.

Detail Description Paragraph - DETX (46):

Cloning of Rabbit Carboxylesterase

Detail Description Paragraph - DETX (77):

[0096] In addition to efficiently converting CPT-11 to the active compound SN-38, experiments were also performed demonstrating the ability of rabbit liver CE to convert the inactive metabolic end product APC to SN-38. No known human enzyme activates APC. FIG. 6 shows the kinetics of conversion of APC to SN-38 by 50 units of rabbit liver CE in an in vitro reaction. FIG. 7 shows that U-373 glioma cells that express the rabbit liver CE, but not human alveolar macrophage carboxylesterase which is 85% homologous to the rabbit enzyme, are sensitized to the growth inhibitory effects of APC. Thus, the combination of APC and sensitization of selected tumor cells with rabbit liver CE as described above can be used to produce a tumor-specific cell death while greatly minimizing the toxic side effects associated with administration of chemotherapy.

PGPUB-DOCUMENT-NUMBER: 20040166115

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040166115 A1

TITLE: Use of multi-specific, non-covalent complexes for targeted delivery of therapeutics

PUBLICATION-DATE: August 26, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 714391

DATE FILED: November 17, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60426379 20021115 US

US-CL-CURRENT: 424/178.1

ABSTRACT:

The invention relates to a method for treating target cells, tissues or pathogens in a subject, comprising administering a non-covalently bound complex which comprises a multispecific targeting protein and a hapten-enzyme covalent conjugate. The invention further relates to kits comprising the non-covalently bound complex or the components to prepare the complex.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. 119(e) to U.S. Provisional Application Serial No. 60/426,379, filed Nov. 15, 2002, the disclosure of which is incorporated by reference herein in its entirety.

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Detail Description Paragraph - DETX (14):

[0041] BsAbs of the types exemplified above can be pre-mixed with several different hapten-enzyme conjugates to produce and deliver an effective therapy agent, after appropriate prodrug administration, depending on what the pertinent arm of the bsAb has been raised against. In a preferred embodiment, the enzyme contained in the hapten-enzyme covalent conjugate is selected from the group consisting of an esterase, carboxylesterase, carboxypeptidase, amidase, glucoronidase and galactosidase. Most preferably, the esterase is a carboxylesterase selected from the group consisting of rat, mouse, rabbit, porcine and human carboxylesterase. The enzyme may be produced by recombinant techniques well know in the art (Wolfe, et al. 1999). The enzyme may be produced in yeast, bacteria, plants, insect or animal cells. Preferably, the enzyme has been modified to enhance its catalytic properties (Wolfe et al, 1999). The modification may be performed via site-directed mutagenesis. See

U.S. Pat. Nos. 5,352,594 and 5,912,161 for a general discussion of site-directed mutagenesis. In any case, the desired effect of the mutagenesis is to reduce the Michaelis constant of the enzyme, enabling more efficient enzyme activity at lower concentrations of prodrug substrate. It is preferred that the multispecific targeting protein binds to both its antigenic target and to its hapten target via the target binding site and the hapten binding site, respectively, with a dissociation constant of at least 10.sup.-7; more preferably at least 10.sup.-9.

Detail Description Paragraph - DETX (19):

[0046] After administration, localization to the site of disease, and substantial. clearance from normal tissues of the bsAb/hapten-enzyme complex, a drug or prodrug substrate to the enzyme in question may be given. For example, with a CEA-expressing tumor, the above MN-14.times.734 bsAb, pre-complexed with DTPA-carboxylesterase is given, allowed to localize to CEA-expressing tumor sites, and clear normal tissues, before the prodrug CPT-11 (irinotecan) (a substrate for carboxylesterase) is given. The non-covalently bound bsAb-hapten-enzyme complex that has localized at the tumor, activates the subsequently administered prodrug specifically at the site of the tumor. A variety of chemotherapeutic agents or prodrugs of chemotherapeutic agents may be used in the practice of the preferred embodiments of the present invention for treatment of subjects. Such chemotherapeutic agents include, but are not limited to, adriamycin, actinomycin, calicheamycin, epothilones, maytansine, mitomycin, carminomycin, daunomycin, doxorubicin, tamoxifen, taxol and other taxanes, taxotere, vincristine, vinblastine, vinorelbine, etoposide (VP-16), 5-fluorouracil (5FU), cytosine arabinoside, cyclophohphamide, thiotepa, methotrexate, camptothecin, actinomycin-D, mitomycin C, cisplatin (CDDP), aminopterin, combretastatin(s), neomycin, and podophyllotoxin(s). Anti-metabolites such as cytosine arabinoside, amethopterin; anthracyclines; vinca alkaloids and other alkaloids; antibiotics, demecolcine; etopside; mithramycin; and other anti-tumor alkylating agents are also contemplated for use in the present invention.

Detail Description Paragraph - DETX (49):

[0074] Two vials of rabbit liver carboxylesterase (about 8.5 mg protein content/vial) are reconstituted with 2.3 mL of 50 mM potassium phosphate buffer pH 7.5, and the solution is made 4.2 mM in DTPA using 0.1 mL of a 0.1 M stock solution of DTPA pH 6.7. The pH of the resultant solution is adjusted to be in the 7.7-7.8 range, and then reacted with 10 mg of cyclic DTPA dianhydride. After 1 h of stirring at the room temperature, the reaction mixture is passed through two successive SEC columns equilibrated in 0.1 M sodium phosphate pH 7.3. The eluate is further purified by preparative HPLC on a TSK G3000SW column using 0.2 M sodium phosphate pH 6.8, at 4 mL/min flow, as the eluent. The purified conjugate is made 0.1 M in sodium phosphate pH 6.8, and concentrated. The DTPA-to-carboxylesterase molar substitution ratio, determined by a metal-binding assay, is estimated to be in the range of 2.95-to-1 to 4.43-to-1.

Detail Description Paragraph - DETX (58):

[0077] Male hamsters (body weight: .about.75 g) are given GW-39 human tumor xenografts by injection of a 20% v/v GW-39 tumor cell suspension intramuscularly on the animals' right thigh. After 3 days, a 2:1 premixed complex of mMN-14 F(ab).sub.2.times.m734Fab' and Indium-DTPA-carboxylesterase, at a dose of 0.75 mg of bsAb, corresponding to 200 enzyme units per kg body weight, is administered. Five days post-injection of bsAb/In-DTPA-carboxylesterase, a maximum tolerated dose (8 mg/.about.75 g body weight; determined earlier) of the prodrug, CPT-11, is given. A positive control group is given CPT-11 alone and an untreated group are also included in the study. Tumor growth in untreated animals is out of control at 3-4 weeks

post-implantation of tumor cells, and animals are sacrificed for humane reasons. Mean tumor volumes are similar for the bsAb/In-DTPA-carboxylesterase and the positive control (CPT-11 alone) at 5 weeks, and out to 9 weeks post-implantation of tumor cells. However, the bsAb/In-DTPA-carboxylesterase treated group continues to show growth inhibition over the next five weeks, while the mean tumor volumes for the group given CPT-11 alone increase during the same period. The relative mean tumor volume for the bsAb/In-DTPA-carboxylesterase treated group at week 14 is similar to the mean tumor volume at week 9 for the positive control, CPT-11 -alone-treated animals. This demonstrates a 5-week advantage in tumor growth control when applying an ADEPT approach using bsAb/In-DTPA-carboxylesterase pretargeting.

Detail Description Paragraph - DETX (78):

[0095] Danks, M. K., Morton, C. L., Krull, E. J., Cheshire, P. J., Richmond, L. B., Naeve, C. W., Pawlik, C. A., Houghton, P. J. and Potter, P. M. Comparison of activation of CPT-11 by rabbit and human carboxylesterases for use in enzyme/prodrug therapy. Clin. Cancer Res., 5:917-924, 1999.

Claims Text - CLTX (18):

17. The method of claim 16, wherein the carboxylesterase is rat, mouse, rabbit, porcine or human carboxylesterase.

US-PAT-NO: 6800483

DOCUMENT-IDENTIFIER: US 6800483 B1

TITLE: Compositions and methods for sensitizing and inhibiting growth of human tumor cells

DATE-ISSUED: October 5, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Potter; Philip M.	Memphis	TN	N/A	N/A
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APPL-NO: 09/ 595682

DATE FILED: June 16, 2000

PARENT-CASE:

This application is a continuation-in-part of PCT/US99/03171 filed Feb. 12, 1999, which claims the benefit of priority from provisional U.S. Application Serial No. 60/075,258, filed Feb. 19, 1998.

US-CL-CURRENT: 435/456, 435/320.1 , 435/325

ABSTRACT:

Polynucleotides encoding carboxylesterase enzymes and polypeptides encoded by the polynucleotides which are capable of metabolizing a chemotherapeutic prodrug and inactive metabolites thereof to active drug are provided. Compositions and methods for sensitizing tumor cells to a prodrug chemotherapeutic agent and inhibiting tumor growth with this enzyme are also provided. In addition, screening assay for identification of drugs activated by this enzyme are described.

4 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

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Brief Summary Text - BSTX (9):

CPT-11 (irinotecan, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin) is a prodrug currently under investigation for the treatment of cancer that is converted to the active drug known as SN-38 (7-ethyl-10-hydroxy-camptothecin) (Tsuji, T. et al. 1991. J. Pharmacobiol. Dynamics 14:341-349; Satoh, T. et al. 1994. Biol. Pharm. Bull. 17:662-664). SN-38 is a potent inhibitor of topoisomerase I (Tanizawa, A. et al. 1994. J. Natl. Cancer Inst. 86:836-842; Kawato, Y. et al. 1991. Cancer Res. 51:4187-4194), an enzyme whose inhibition in cells can result in DNA damage and induction of apoptosis (Hsiang, Y. -H. et al. 1989. Cancer Res. 49:5077-5082). The specific enzyme responsible for activation in

vivo of CPT-11 has not been identified, although serum or liver homogenates from several mammalian species have been shown to contain activities that convert CPT-11 to SN-38 (Tsuji, T. et al. 1991. J. Pharmacobiol. Dynamics 14:341-349; Senter, P. D. et al. 1996. Cancer Res. 56:1471-1474; Satoh, T. et al. 1994. Biol. Pharm. Bull. 17:662-664). Uniformly, these activities have characteristics of carboxylesterase (CE) enzymes (Tsuji, T. et al. 1991. J. Pharmacobiol. Dynamics 14:341-349; Senter, P. D. et al. 1996. Cancer Res. 56:1471-1474; Satoh, T. et al. 1994. Biol. Pharm. Bull. 17:662-664). In fact, SN-38 can be detected in the plasma of animals and humans minutes after the administration of CPT-11 (Stewart, C. F. et al. 1997. Cancer Chemother. Pharmacol. 40:259-265; Kaneda, N. et al. 1990. Cancer Res. 50:1715-1720; Rowinsky, E. K. et al. 1994. Cancer Res. 54:427-436), suggesting that a CE enzyme present in either serum or tissues can convert the camptothecin analog to its active metabolite.

Brief Summary Text - BSTX (18):

In the present invention, polynucleotides encoding carboxylesterase enzymes or active fragments thereof and polypeptides encoded thereby which are capable of metabolizing the chemotherapeutic prodrug CPT-11 and its inactive metabolite APC to active drug SN-38 are disclosed. Use of these enzymes in combination with APC renders this inactive metabolite a useful chemotherapeutic prodrug. It has also been found that compositions comprising a polynucleotide of the present invention and a disease-specific responsive promoter can be delivered to selected tumor cells to sensitize the tumor cells to the chemotherapeutic prodrug CPT-11, thereby inhibiting tumor cell growth.

Drawing Description Text - DRTX (2):

FIG. 1 shows the alignment of the amino acid sequences of a rabbit liver carboxylesterase (Rab; GenBank Accession # AF036930), a human liver carboxylesterase (hCE1; GenBank Accession # M73499) and the human intestinal carboxylesterase (hiCE; GenBank Accession # Y09616). The active site triad (Ser-240, Glu-364 and His-478) are indicated by an asterisk (*). Identical residues are indicated by a vertical line (.vertline.), conservative changes by a colon (:), semi-conservative changes by a period (.), and computer inserted gaps within the amino acids are indicated by a dash (-). Large areas of homology between all three proteins are shaded.

Drawing Description Text - DRTX (6):

FIG. 4 shows the complete coding sequence of the rabbit liver CE (SEQ ID NO:20) and the amino acid sequence encoded thereby (SEQ ID NO:21). The 1698 bp ORF encodes a 62.3 kDa protein. The N-terminal hydrophobic leader sequence is in italics, the 5' and 3' RACE sequences are underlined and the potential active site serine is indicated by an asterisk. The carboxylesterase B-1 and B-2 motifs, at amino acids 208-223 and 114-124 are double underlined. Numbers over the sequence refer to nucleotide position whereas numbers along the left margin refer to amino acid residues.

Detailed Description Text - DETX (2):

CPT-11 is a promising anti-cancer prodrug, that when given to patients, is converted to its active metabolite SN-38 by a human carboxylesterase. However, conversion in patients is relatively inefficient and less than 5% of the prodrug is metabolized to SN-38 (Rivory, L. P. et al. 1997. Clin. Cancer Res. 3:1261-1266). In patients, this prodrug is also metabolized to APC (Haaz, M-C. et al. 1998. Cancer Res. 58:468-472). APC has little, if any, active anti-tumor activity and is not converted to an active metabolite in humans (Rivory, L. P. et al. 1996. Cancer Res. 56:3689-3694). Accordingly, high concentrations of this prodrug must be administered to achieve effective levels of active drug in vivo. However, myelosuppression and secretory diarrhea limit the amount of prodrug that can be administered to patients.

Detailed Description Text - DETX (4):

In accordance with one aspect of the present invention there are provided polynucleotides which encode carboxylesterases capable of metabolizing a chemotherapeutic prodrug and inactive metabolites thereof to active drug. By "polynucleotides" it is meant to include any form of DNA or RNA such as cDNA or genomic DNA or MRNA, respectively, encoding these enzymes or an active fragment thereof which are obtained by cloning or produced synthetically by well known chemical techniques. DNA may be double- or single-stranded. Single-stranded DNA may comprise the coding or sense strand or the non-coding or antisense strand. Thus, the term polynucleotide also includes polynucleotides which hybridize under stringent conditions to the above-described polynucleotides. As used herein, the term "stringent conditions" means at least 60% homology at hybridization conditions of 60.degree. C. at 2.times.SSC buffer. In one embodiment, the polynucleotide comprises the cDNA depicted in FIG. 4 (SEQ ID NO:20) or a homologous sequence or fragment thereof which encodes a polypeptide having similar activity to that of this rabbit liver CE enzyme. In another embodiment, the polynucleotide comprises a cDNA as depicted in SEQ ID NO:27 encoding human intestinal carboxylase as depicted in SEQ ID NO:28. Due to the degeneracy of the genetic code, polynucleotides of the present invention may also comprise other nucleic acid sequences encoding these enzymes and derivatives, variants or active fragments thereof. The present invention also relates to variants of these polynucleotides which may be naturally occurring, i.e., allelic variants, or mutants prepared by well known mutagenesis techniques.

Detailed Description Text - DETX (25):

Another aspect of the present invention relates to the ability of compositions comprising a polynucleotide encoding a carboxylesterase and a disease-specific responsive promoter of selected tumor cells to sensitize the tumor cells to a chemotherapeutic prodrug. The ability of a rabbit CE or a human intestinal CE of the present invention to sensitize human tumor cells to CPT-11 was examined. Experiments were first performed to confirm that the metabolite produced by the activity of a CE of the present invention is biologically active in vitro. Rh30 cells were exposed to the products of each reaction for one hour and the percentage of growth inhibition was determined. As expected, Rh31 cells exposed to 1 to 5 units of CE that had been inactivated by heating produced no inhibition of cell growth. In contrast, reaction products of CPT-11 incubated with 1 to 5 units of active CE produced a 30-60% inhibition of cell growth. These data are consistent with the conversion of CPT-11 to SN-38 by CE in these cells. Similar confirmatory experiments were performed with COS-7 cells.

Detailed Description Text - DETX (44):

The rabbit proteins were subjected to automated N-terminal amino acid sequencing. Both bands yielded protein sequences indicating that the peptides were not N-terminally blocked. The derived amino acid sequences were analyzed by computer searches using the Fasta and BLAST comparison programs.. Band 1 (approximately 60 kDa) demonstrated significant homology with several CE sequences, including a rabbit CE, present in the GenBank and Swissprot databases (FIG. 1). However, the nucleic acid sequence encoding rabbit CE protein has not been disclosed. In addition, comparison of the amino acid sequence of the polypeptide encoded by the cDNA of the present invention with the published amino acid sequence for rabbit CE showed three mismatches. Further, the polypeptide encoded by the CDNA of the present invention contains an 8 amino acid insert and an 18 amino acid leader sequence at the N-terminus which the published sequence does not contain. Thus, the published amino acid sequence of a rabbit liver carboxylesterase protein (Swissprot Accession Number P12337; Korza, G. and J. Ozols. 1988. J. Biol. Chem. 263:3486-3495) is

different from the polypeptide encoded by the cDNA of the present invention.

Detailed Description Text - DETX (47):
Cloning of Rabbit Carboxylesterase

Detailed Description Text - DETX (78):
In addition to efficiently converting CPT-11 to the active compound SN-38, experiments were also performed demonstrating the ability of rabbit liver CE to convert the inactive metabolic end product APC to SN-38. No known human enzyme activates APC. FIG. 6 shows the kinetics of conversion of APC to SN-38 by 50 units of rabbit liver CE in an in vitro reaction. FIG. 7 shows that U-373 glioma cells that express the rabbit liver CE, but not human alveolar macrophage carboxylesterase which is 85% homologous to the rabbit enzyme, are sensitized to the growth inhibitory effects of APC. Thus, the combination of APC and sensitization of selected tumor cells with rabbit liver CE as described above can be used to produce a tumor-specific cell death while greatly minimizing the toxic side effects associated with administration of chemotherapy.

Claims Text - CLTX (1):
1. A method for sensitizing tumor cells to a chemotherapeutic prodrug APC or CPT-11 in vitro comprising transfecting selected tumor cells with a composition comprising an isolated polynucleotide encoding a carboxylesterase wherein said carboxylesterase is operably linked to a promoter that directs expression of said carboxylesterase in said tumor cells, and wherein expression of the carboxylesterase renders the tumor cells more sensitive to the cytotoxic effect of said chemotherapeutic prodrug APC or CPT-11.

Claims Text - CLTX (3):
3. The method according to claim 1 wherein the carboxylesterase is selected from the group consisting of rabbit carboxylesterase and human intestinal carboxylesterase.

Other Reference Publication - OREF (11):
Pawlik et al., "Use of the Ornithine Decarboxylase Promoter to Achieve N-MYC-Mediated Overexpression of a Rabbit Carboxylesterase to Sensitize Neuroblastoma Cells to CPT-11", Molecular Therapy 2000 1(5):457-463.

Other Reference Publication - OREF (12):
Meck et al., "A Virus-directed Enzyme Prodrug Therapy Approach to Purging Neuroblastoma Cells from hematopoietic Cells Using Adenovirus Encoding Rabbit Carboxylesterase and CPT-11.sup.1 ", Cancer Research 2001 61:5083-5089.

Other Reference Publication - OREF (14):
Wadkins et al., "Structural Constraints Affect the metabolism of 7-Ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (CPT-11) by Carboxylesterases", Mol. Pharmocol. 2001 60(2):355-362.

Other Reference Publication - OREF (25):
Humerickhouse et al., "Characterization of CPT-11 Hydrolysis by Human Liver Carboxylesterase Isoforms hCE-1 and hCE-2.sup.1 ", 2000 Cancer Res. 60:1189-1192.

Other Reference Publication - OREF (45):
Satoh, T., et al., "Metabolic Activation of CPT-11, 7-Ethyl-10-[4-1(piperidino)-1-piperidino]carbonyloxycamptothecin, a Novel Antitumor Agent, by Carboxylesterase" 1994 Biol. Pharm. Bull. 17:662-664.

Other Reference Publication - OREF (46):

Senter, P.D., et al., "The Role of Rat Serum Carboxylesterase in the Activation of Paclitaxel and Camptothecin Prodrugs", 1996 Cancer Res. 56:1471-1474.

US-PAT-NO: 6770632

DOCUMENT-IDENTIFIER: US 6770632 B1
See image for Certificate of Correction

TITLE: Folypolyglutamyl synthetase gene transfer to enhance antifolate

DATE-ISSUED: August 3, 2004

INVENTOR-INFORMATION:

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Breakefield; Xandra O.	Newton	MA	N/A	N/A

APPL-NO: 09/ 617116

DATE FILED: July 14, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/144,074, filed on Jul. 16, 1999, which is herein incorporated by reference.

US-CL-CURRENT: 514/44, 424/93.2 , 435/320.1 , 435/455 , 435/456 , 435/458 , 435/459

ABSTRACT:

. Methods of killing neoplastic cells are provided. The invention relates to the use of folylpolyglutamyl synthetase (FPGS) gene transfer to enhance the sensitivity of several types of tumor cells to polyglutamylatable antifolate drugs, such as methotrexate (MTX) and edatrexate (EDX).

21 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

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Detailed Description Text - DETX (74):
One criterion by which a drug-enhancing gene therapy can be evaluated is the enhancement of drug sensitivity seen in clones resulting from transfection of a tumor cell line with the cDNA for the drug-activating enzyme. The magnitude of this enhancement can be quantified by the shift in ED.sub.50 observed between nontransfected and transected tumor cell lines. When cDNAs encoding for herpes simplex virus thymidine kinase (HSV-TK) and E. coli cytosine deaminase (CD) were stably transfected into 9L cells, the ED.sub.5 shifts were 500-fold for ganciclovir treatment and 80-fold for 5-fluorocytosine treatment (Aghi, M., et al., Journal of the National Cancer Institute 90:370-380 (1998)). The 67-fold shift in ED.sub.50 observed with EDX treatment of 9L and 9L/FPGS cells in this

report is close to the shift observed for the cytosine deaminase prodrug-activating system in 9L cells. And it represents a better enhancement of sensitivity than the 8-fold decrease in ED.sub.50 obtained when a rhabdomyosarcoma cell line was transfected with the rabbit carboxylesterase cDNA and treated with the prodrug CPT-11 (Danks, M. K., et al., Cancer Research 58:20-22 (1998)). Relatively large enhancement in drug sensitivity is achievable after delivery of genes like HSV-TK and CD partially because these genes are microbial, making tremendous increases in relative expression in mammalian tumors feasible. FPGS is a mammalian gene expressed to a certain extent by mammalian tumors, making relative increases in expression harder to achieve. However, two advantages of the slight FPGS expression and slight antifolate sensitivity found in tumors that would be candidates for FPGS gene transfer are: (1) tumor cells expressing foreign enzymes like HSV-TK and CD can be killed by an immune response before generating enough active drug to mediate a bystander effect, while tumor cells expressing FPGS encounter no such response; and (2) transgene expression often shuts down over time during gene therapy--loss of HSV-TK or CD expression would render ganciclovir or 5-fluorocytosine completely ineffective, while loss of expression of delivered FPGS would leave behind native FPGS activity, allowing antifolates to retain a slight anticancer effect (Roth, J. A., Cristiano, R. J., Journal of the National Cancer Institute 89:21-39 (1997)).

Other Reference Publication - OREF (25):

Danks, M.K. et al., "Overexpression of a Rabbit Liver Carboxylesterase Sensitizes Human Tumor Cells to CPT-11," Cancer Res. 58:20-22 (Jan. 1998).

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FILE 'HOME' ENTERED AT 08:28:51 ON 02 MAY 2005

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COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
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FULL ESTIMATED COST                                         0.21       0.21

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FILE 'SCISEARCH'
L2         1545 CARBOXYLESTERASE#

FILE 'LIFESCI'
L3          735 CARBOXYLESTERASE#

FILE 'BIOTECHDS'
L4          218 CARBOXYLESTERASE#

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L5         1835 CARBOXYLESTERASE#

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L7         2680 CARBOXYLESTERASE#

FILE 'NTIS'
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FILE 'CANCERLIT'
L12         244 CARBOXYLESTERASE#

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=> s cpt-11 or irinotecan
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                  (CPT(W)11)
           2462 IRINOTECAN
L14        2644 CPT-11 OR IRINOTECAN
```

```
FILE 'SCISEARCH'
          5146 CPT
        485301 11
          1495 CPT-11
                 (CPT(W)11)
          2177 IRINOTECAN
L15       2627 CPT-11 OR IRINOTECAN

FILE 'LIFESCI'
           611 "CPT"
         75326 "11"
            77 CPT-11
                 ("CPT"(W)"11")
            65 IRINOTECAN
L16        115 CPT-11 OR IRINOTECAN

FILE 'BIOTECHDS'
            74 CPT
         34993 11
            24 CPT-11
                 (CPT(W)11)
            56 IRINOTECAN
L17         69 CPT-11 OR IRINOTECAN

FILE 'BIOSIS'
          4058 CPT
        515514 11
          1193 CPT-11
                 (CPT(W)11)
          1619 IRINOTECAN
L18       2127 CPT-11 OR IRINOTECAN

FILE 'EMBASE'
          4689 "CPT"
        362786 "11"
          1897 CPT-11
                 ("CPT"(W)"11")
          5240 IRINOTECAN
L19       5290 CPT-11 OR IRINOTECAN

FILE 'HCAPLUS'
          4939 CPT
        873686 11
          1030 CPT-11
                 (CPT(W)11)
          1737 IRINOTECAN
L20       2235 CPT-11 OR IRINOTECAN

FILE 'NTIS'
           561 CPT
         73816 11
             1 CPT-11
                 (CPT(W)11)
             1 IRINOTECAN
L21          1 CPT-11 OR IRINOTECAN

FILE 'ESBIOBASE'
          1991 CPT
        152893 11
           628 CPT-11
                 (CPT(W)11)
           963 IRINOTECAN
L22       1180 CPT-11 OR IRINOTECAN
```

```
FILE 'BIOTECHNO'
           899 CPT
         86517 11
           311 CPT-11
                 (CPT(W)11)
           651 IRINOTECAN
L23        675 CPT-11 OR IRINOTECAN

FILE 'WPIDS'
           418 CPT
       1544566 11
           124 CPT-11
                 (CPT(W)11)
           318 IRINOTECAN
L24        393 CPT-11 OR IRINOTECAN

FILE 'CANCERLIT'
          1751 CPT
        153379 11
          1160 CPT-11
                 (CPT(W)11)
          1549 IRINOTECAN
L25       1831 CPT-11 OR IRINOTECAN

TOTAL FOR ALL FILES
L26      19187 CPT-11 OR IRINOTECAN

=> s l13 and l26
FILE 'MEDLINE'
L27          99 L1 AND L14

FILE 'SCISEARCH'
L28        138 L2 AND L15

FILE 'LIFESCI'
L29          8 L3 AND L16

FILE 'BIOTECHDS'
L30          7 L4 AND L17

FILE 'BIOSIS'
L31        107 L5 AND L18

FILE 'EMBASE'
L32        121 L6 AND L19

FILE 'HCAPLUS'
L33        107 L7 AND L20

FILE 'NTIS'
L34          0 L8 AND L21

FILE 'ESBIOBASE'
L35         75 L9 AND L22

FILE 'BIOTECHNO'
L36         40 L10 AND L23

FILE 'WPIDS'
L37          6 L11 AND L24

FILE 'CANCERLIT'
L38         66 L12 AND L25
```

```
TOTAL FOR ALL FILES
L39         774 L13 AND L26

=> s l39 and py<=1999 range=2004,
FILE 'MEDLINE'
'2004,' IS NOT A VALID RANGE FOR FILE 'MEDLINE'
SEARCH ENDED BY USER

FILE 'SCISEARCH'
            36 PY<=1999
L40          0 L28 AND PY<=1999

FILE 'LIFESCI'
           462 PY<=1999
L41          0 L29 AND PY<=1999

FILE 'BIOTECHDS'
             8 PY<=1999
                 (PY<=1999)
L42          0 L30 AND PY<=1999

FILE 'BIOSIS'
           542 PY<=1999
L43          0 L31 AND PY<=1999

FILE 'EMBASE'
           200 PY<=1999
L44          0 L32 AND PY<=1999

FILE 'HCAPLUS'
         13888 PY<=1999
L45          1 L33 AND PY<=1999

FILE 'NTIS'
          1594 PY<=1999
L46          0 L34 AND PY<=1999

FILE 'ESBIOBASE'
             0 PY<=1999
L47          0 L35 AND PY<=1999

FILE 'BIOTECHNO'
       1285569 PY<=1999
L48         19 L36 AND PY<=1999

FILE 'WPIDS'
          1302 PY<=1999
                 (PY<=1999)
L49          0 L37 AND PY<=1999

FILE 'CANCERLIT'
             0 PY<=1999
L50          0 L38 AND PY<=1999

TOTAL FOR ALL FILES
L51         20 L39 AND PY<=1999

=> d l45

L45  ANSWER 1 OF 1  HCAPLUS  COPYRIGHT 2005 ACS on STN
TI   Rabbit liver carboxylesterase capable of activating
     chemotherapeutic prodrug and thereby sensitizing and inhibiting growth of
     human tumor cells
SO   U.S., 39 pp., Cont.-in-part of WO 99 42,593.
```

```
     CODEN: USXXAM
IN   Danks, Mary K.; Potter, Philip M.; Houghton, Peter J.
AN   2004:817401  HCAPLUS
DN   141:289026
     PATENT NO.          KIND   DATE        APPLICATION NO.         DATE
     --------------      ----   --------    --------------------    --------
PI   US 6800483           B1    20041005    US 2000-595682          20000616
     WO 9942593           A1    19990826    WO 1999-US3171          19990212 <--
         W:  AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE,
             DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP,
             KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN,
             MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
             TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU,
             TJ, TM
         RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES,
             FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI,
             CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
     US 2004259829         A1    20041223    US 2004-858271          20040601


=> fil medl
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      19.01      19.22

FILE 'MEDLINE' ENTERED AT 08:31:59 ON 02 MAY 2005

=> s 139 and py<=1999 range=2004000000,
         18530 PY<=1999
L52          1 L27 AND PY<=1999

=> d

L52  ANSWER 1 OF 1     MEDLINE on STN
TI   Pharmacology of irinotecan.
SO   Drugs of today (Barcelona, Spain : 1998), (1998 Sep) 34 (9)
     777-803.
     Journal code: 101160518. ISSN: 0025-7656.
AU   Robert J; Rivory L
AN   2004097687     MEDLINE


=> fil .becpat
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.43      19.65

FILES 'BIOTECHDS, HCAPLUS, WPIDS' ENTERED AT 08:32:34 ON 02 MAY 2005
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

3 FILES IN THE FILE LIST

=> s 139 and wo/pc and pry<=1999 range=2004,
FILE 'BIOTECHDS'
          8593 WO/PC
           397 PRY<=1999
                 (PRY<=1999)
L53          0 L30 AND WO/PC AND PRY<=1999

FILE 'HCAPLUS'
         73562 WO/PC
         43959 PRY<=1999
L54          2 L33 AND WO/PC AND PRY<=1999
```

```
FILE 'WPIDS'
        154034 WO/PC
         33759 PRY<=1999
                (PRY<=1999)
L55           1 L37 AND WO/PC AND PRY<=1999

TOTAL FOR ALL FILES
L56           3 L39 AND WO/PC AND PRY<=1999

=> dup rem l56
PROCESSING COMPLETED FOR L56
L57            2 DUP REM L56 (1 DUPLICATE REMOVED)

=> d tot

L57  ANSWER 1 OF 2  HCAPLUS  COPYRIGHT 2005 ACS on STN DUPLICATE 1
TI   Anti-CD74 immunoconjugates and their therapeutic and diagnostic uses
SO   U.S. Pat. Appl. Publ., 44 pp., Cont.-in-part of U.S. Ser. No. 377,122.
     CODEN: USXXCO
IN   Griffiths, Gary L.; Hansen, Hans J.; Goldenberg, David M.; Lundberg, Bo B.
AN   2004:934160  HCAPLUS
DN   141:388650
     PATENT NO.           KIND   DATE        APPLICATION NO.         DATE
     ---------------      ----   --------    --------------------    --------
PI   US 2004219203         A1    20041104    US 2003-706852          20031112 <--
     US 6306393            B1    20011023    US 1999-307816          19990510 <--
     US 2002071807         A1    20020613    US 2001-965796          20011001 <--
     US 2003124058         A1    20030703    US 2002-314330          20021209 <--
     US 2003133930         A1    20030717    US 2003-350096          20030124 <--
     US 2004115193         A1    20040617    US 2003-377122          20030303
     WO 2004110390         A2    20041223    WO 2004-US19238         20040617 <--
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
             CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
             GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
             LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI,
             NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY,
             TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
         RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM,
             AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK,
             EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE,
             SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE,
             SN, TD, TG

L57  ANSWER 2 OF 2  HCAPLUS  COPYRIGHT 2005 ACS on STN
TI   Rabbit liver carboxylesterase capable of activating
     chemotherapeutic prodrug and thereby sensitizing and inhibiting growth of
     human tumor cells
SO   U.S., 39 pp., Cont.-in-part of WO 99 42,593.
     CODEN: USXXAM
IN   Danks, Mary K.; Potter, Philip M.; Houghton, Peter J.
AN   2004:817401  HCAPLUS
DN   141:289026
     PATENT NO.           KIND   DATE        APPLICATION NO.         DATE
     ---------------      ----   --------    --------------------    --------
PI   US 6800483            B1    20041005    US 2000-595682          20000616 <--
     WO 9942593            A1    19990826    WO 1999-US3171          19990212 <--
         W:  AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE,
             DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP,
             KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN,
             MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
             TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU,
             TJ, TM
         RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES,
             FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI,
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CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
US 2004259829 A1 20041223 US 2004-858271 20040601 <--

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	10.17	29.82

STN INTERNATIONAL LOGOFF AT 08:33:45 ON 02 MAY 2005